

US EPA ARCHIVE DOCUMENT

MRID 43035401

**DATA EVALUATION RECORD**  
**72-4(B) FRESHWATER FLOW-THROUGH INVERTEBRATE LIFE CYCLE TEST**

1. **CHEMICAL:** Methyl Parathion Shaughnessey #: 053501
2. **TEST MATERIAL:** Technical Purity: 95.7%
3. **CITATION:**

Author: Jennifer W. Blasberg, Hugh Murrell, and John Bucksath  
Title: Chronic Toxicity of Methyl Parathion to *Daphnia magna* Under Flow-Through Test Conditions  
Date: 10/20/93  
Laboratory Report #: 40350  
Any Other Study #: N/A  
Sponsor: Cheminova Agro A/S  
Sponsor #: N/A  
Laboratory: Analytical Bio-Chemistry Laboratories, Inc  
MRID No.: 43035401

4. **REVIEWED BY:**

Dennis J. McLane, Wildlife Biologist  
Ecological Effects Branch (7507C)  
U.S.E.P.A.

Signature: *Dennis J. McLane*  
Date: 10-4-94

5. **APPROVED BY:**

Les W. Touart  
Section Head  
Ecological Effects Branch (7507C)  
U.S.E.P.A.

Signature: *L. W. Touart*  
Date: 10/6/94

6. **CONCLUSIONS:** This study scientifically sound but does not fulfill the guideline requirement for a life-cycle study with *Daphnia magna* a freshwater aquatic invertebrate. The NOEC is 0.43 µg/L and the LOEC is 0.85 µg/L for survival, weight, and first brood day. This would place methyl parathion in the very highly toxic category. NOEC was not determined for young/adult/reproductive day and length.

7. **MAJOR GUIDELINE DEVIATIONS:**

- (1) Approximately for 3 days the D.O. was below 50% at the nominal concentration of 2 µg/L. However, the daphnids had died by that time.
- (2) The production of young was not reported for various



time periods.

(3) Measured concentrations prior to mixing were not reported.

(4) The measured concentrations for this comparison between replicates was not reported.

(5) The study did not provide adequate information to show that a solvent was needed. The water solubility of methyl parathion is 50 to 60 ppm and the highest level tested was 2 ug/L or 0.002 ppm.

(6) The solvent control was statistically different from the control. Therefore, the effects of the methyl parathion may be masked are difficult to impossible to determine.

## 8. MATERIALS AND METHODS:

### A. Biological System:

Guideline Criteria	Reported Information
<b>Species:</b> <i>Daphnia</i>	<i>Daphnia magna</i>
<b>Source</b>	ABC Laboratories' in-house culture
<b>Food variety</b> 5 mg/L dry weight - 1) Synthetic (trout chow); 2) Alfalfa yeast and algae; 3) Algae <i>Ankistrodesmus falcatus</i> ; <i>Chlamydomonas reinhardtii</i> ; and <i>Selenastrum capricornutum</i> $10^8$ algal cells per L 4); 4) Solution be made up < 4 hours before test begins	1) Yes 2) Yes 3) <i>Selenastrum capricornutum</i> / <i>Ankistrodesmus falcatus</i> ; $3 \times 10^8$ cells/mL per Liter of test solution and trout chow 4) Not reported
<b>Age at beginning of test:</b> 1) At least 10-12 days old (those that have had at least on brood) should be separated from the culture, 2) Put in a separate culture container and maintained for at least 21 days to insure that good health and conditions are present.	1) Yes 2) Not reported

Guideline Criteria	Reported Information
<b>Renewal:</b> 1) Renewal schedule Monday, Wednesday, and Friday must be set-up for counting; 2) Parent daphnids in all beakers counted; 3) 7 beakers containing 1 parent daphnid each, the offspring, both live and dead, are counted and discarded.	1) Counted offspring  2) Yes  3) N/A
<b>Duration:</b> on 21 <sup>st</sup> day 1) Count parents 2) Measure to nearest 0.01 mm from apex of helmet to the base of the spine. 3) # young, both alive and dead.	1) Yes 2) Yes, reported to 0.01 mm  3) "...the offspring produced.."
<b>Test Rejection:</b> 1) 30 % die; 2) Must $\geq$ 40 young after 21 days; 3) Production of "resting eggs" or ephippia; 4) Temp. changes over 5°C from 20°C in 48 hrs; 5) DO >50% for 48 hrs;  6) pH changes > one unit in 48 hrs.	1) No 2) Yes  3) No ephippia reported 4) No changes over 1°C 5) From day 17 until day 21 the D.O. was below (see calculations- figure 1 and graph 1) 6) No changes over 4/10 of a unit
<b>Data Endpoints:</b> 1) Survival of 1 <sup>st</sup> generation . 2) Production of young at various times for each treatment 3) Length of 1 <sup>st</sup> generation at end of test	1) Yes 2) No  3) Yes

Comments: None

**B. Physical System:**

Guideline Criteria	Reported Information
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<b>Test Water:</b> 1) Well or spring provided that the source is not polluted 2) Tested for contaminants  3) Measured pH 7.6 and 8.0. 4) Hardness 160 to 180 mg/L 5) Reconstituted water detailed descriptions (ASTM)	1) well water and well water reverse osmosis water  2) Yes, Boron-0.259 ppm; Fluoride-0.65 ppm; Lead-0.0024 ppm 3) 8.0-8.4 pH 4) 126-148 mg/L 5) N/A
<b>Test Temperature:</b> 20°C±2°C	21-22°C
<b>Photoperiod:</b> 1) Recommend 16L/8D; 2) Intensity should be 400 to 800 Lux (37 to 74 footcandles) at surface with wide-spectrum fluorescent lamps.	1) 16L/8D 2) 625-726 lux (58-67 footcandles) by cool-white fluorescent bulbs.
<b>Dosing Apparatus:</b> 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.	1) A half-liter diluter system described by Mount and Brungs  2) 5 levels- 0.12, 0.24, 0.5, 1.0, 2.0 µg/L
<b>Toxicant Mixing:</b> 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	1) Yes 2) No  3) Measured concentrations were not made prior to mixing  4) The measured concentrations for this comparison could not be found.

<b>Test Vessels:</b> 1) All glass, No. 316 stainless steel, or perfluorocarbon plastics; volume 200 mls. 250 ml (borosilicate glass beakers are typically used). 2) Vessels should be covered with glass plates to prevent evaporation.	1) Test chambers 1-L glass beakers; 50-mesh stainless steel screens; the material in the diluter not reported  2) Not reported
<b>Retention and Pairing Chambers:</b> Glass with mesh nylon or stainless steel screens	Yes
<b>Flow Rate:</b> 1) Flow rates should provide 5 to 10 volume additions per 24 hr; 2) Flow rate must maintain D.O. at or above 60% of saturation and maintain the toxicant level.	1) 1 L test volume 7.0 times in 24-hours  2) D.O. below 60% Once on day 7 @ 2 µg/L; Once on day 14 @ 1 µg/L; Four times on day 21 sol. control, 0.5, 1.0, and 2.0 µg/L. (see EEB Figure 1)
<b>Aeration:</b> 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks should not be aerated.	1) (see 2) above under Flow Rate:)  2) Not aerated

Comments: No comments.

**C. Chemical System:**

Guideline Criteria	Reported Information
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<p><b>Concentrations:</b></p> <p>1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <p>2) Toxicant conc. must be measured in one tank at each toxicant level every week.</p> <p>3) Food conc. dry weight 5 mg/L for synthetic; algal cells <math>10^8</math></p>	<p>1) Yes, control, sol. control, 0.12, 0.24, 0.5, 1.0, 2.0 <math>\mu\text{g/L}</math></p> <p>2) Composite of 4 beakers at each level</p> <p>3) Trout chow and yeast = final suspended solids conc. of 5 mg/mL per chamber; <i>Selenastrum capricornutum</i>/<i>Ankistrodesmus falcatus</i>; <math>3 \times 10^8</math> cells/mL per Liter of test solution</p>
<p><b>Other Variables:</b></p> <p>1) D.O. must be measured at each conc. at least once a week;</p> <p>2) Freshwater parameters in a control &amp; 1 conc. must be analyzed 1 a week; (pH, alkalinity, hardness, and conductance</p> <p>3) pH should be measured weekly.</p>	<p>1) "...days 0, 4, 7, 14, 21 in alternating duplicate replicates (test chambers A and C on days 0, 7, and 21 and test chambers B and D on days 4 and 14) of the control, vehicle blank, and all test concentrations."</p> <p>2) "Water quality parameters of temperatures, hardness, dissolved oxygen, pH, alkalinity, and conductivity were measured daily (Monday-Friday) for the duration of the study.</p> <p>3) see 2) above.</p>
<p><b>Solvents:</b></p> <p>1) Should not exceed 0.1 ml/L in a flow-through system.</p> <p>2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.</p>	<p>1) 0.025 mL/L</p> <p>2) Dimethylformamide (DMF)</p>

Comments: No comments.

**9. REPORTED RESULTS:****A. Required Endpoints:**

Guideline Criteria	Reported Information
<b>Data Endpoints</b> must include: 1) Survival of first-generation daphnids 2) Number of live young produced per female 3) Dry weight and length of each first-generation daphnids alive at the end of the test	1) see Table IV 2) see Table V 3) see Table VI
<b>Raw data included?</b> (Y/N)	Y

**B. Effects Data:** (The following is excerpted from the study report Table IX)

Mean Measured Conc.	Mean % Survival	Mean YAD	Mean Length (mm)	Mean Weight (mg)	1 <sup>st</sup> BRD Day
Control	97.5	7.83	3.83	0.47	7
Vehicle Blank (DMF)	87.5	13.0	4.44	0.78	7
Pooled Controls	92.5	NA	NA	NA	7
(0.076) $\mu\text{g/L}$	100	9.36V	4.11V	0.64	7
(0.23) $\mu\text{g/L}$	97.5	11.5V	4.33	0.77	7
(0.43) $\mu\text{g/L}$	92.5	12.2	4.42	0.7	7
(0.85) $\mu\text{g/L}$	80CP	10.6V	4.28V	0.62V	7
(1.7) $\mu\text{g/L}$	0CV	7.5*	NA	NA	7



Control and vehicle blank were compared by a Student t test and found to be significantly different for the following parameters: length, weight, and young/adult/reproductive day (YAD). Data subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple means comparison test. Significant differences to the control, vehicle blank, and pooled controls are noted with a C, V, and P, respectively.

Key to abbreviations; NA = Not Applicable, YAD = Young/adult/reproductive day, and BRD = Brood.

\*Level 5 not used in analysis because it was affected for survival.

C. Toxicity Observations: See attached Tables

D. Statistical Results:

(1) Statistical Method: ANOVA AND Dunnett's

NOEC: <0.076 µg/L LOEC: N/A

Endpoint: Young /Adult/ Reproductive Days

(2) Statistical Method: ANOVA AND Dunnett's

NOEC: <0.076 µg/L LOEC: N/A

Endpoint: Day 21 Daphnid Length

(3) Statistical Method: ANOVA AND Dunnett's

NOEC: 0.43 µg/L LOEC: 0.85 µg/L

Endpoint: Mean Weight

(4) Statistical Method: ANOVA AND Dunnett's

NOEC: 0.43 µg/L LOEC: 0.85 µg/L

Endpoint: Mean % Survival

E. Comments: The statistic method used for length is not correct. The Toxstat program warnings that: "This data set has unequal replicates. The Bonferroni T-test should be used instead of the Dunnett's test. As shown below the under (2) did not show that any level was statistical different from the other:

10. Reviewer's Statistical Results:

(1) Statistical Method: ANOVA and Dunnett's

NOEC: N/A LOEC: N/A

Endpoint: Young /Adult/ Reproductive Days

(2) Statistical Method: ANOVA and Bonferroni's

NOEC: N/A LOEC: N/A

Endpoint: Day 21 Daphnid Length

(3) Statistical Method: ANOVA and Dunnett's

NOEC: 0.43  $\mu\text{g/L}$  LOEC: 0.85  $\mu\text{g/L}$

Endpoint: Mean Weight

(4) Statistical Method: ANOVA and Dunnett's

NOEC: 0.43  $\mu\text{g/L}$  LOEC: 0.85  $\mu\text{g/L}$

Most sensitive endpoint: Survival

Comments: The study reports:

" Statistical comparison with the vehicle blank for effects on survival, adult mean length, adult mean weight, days to first brood, and young/adult/reproductive day resulted in an NOEC for survival at level 4 (0.85  $\mu\text{g/L}$ ), NOEC for days to first brood at level 5 (1.7  $\mu\text{g/L}$ ), and NOECs for length, weight, and YAD at level 3 (0.43  $\mu\text{g/L}$ ), although some statistical differences were noted at lower concentrations. These differences are directly attributable to the solvent enhancements effects on the vehicle blank group and decreasing quantities of solvent in the lower test concentrations. For both reproduction and the growth parameters (length and weight), daphnids in the vehicle blank group showed enhancements when compared to the dilution water control group; growth and reproduction in the dilution water control group were within normal range (Table X). For growth and reproduction, the data indicated a general dose-related trend of increased values with increasing solvent concentration until methyl parathion increased to toxic levels."

EEB does not believe that this study provides enough

information to show that DMF caused the erratic YAD and length responses. Based on young/adult/reproductive<sup>day</sup>, the study failed to produce an NOEC. The 0.075 µg/L, 0.23 µg/L and 0.85 µg/L levels were different from the solvent control but the 0.43 µg/L was not. The 0.43 µg/L had DMF and should have provided the same opportunity for increase production as the lower concentration. Similarly with length the 0.076 µg/L and the two levels in between do not show effects.

**11. ADEQUACY OF THE STUDY:**

A. Classification: Supplemental

B. Rationale: NOEC was not established for young/adult/reproductive day and length.

C. Reparability: Repeat the study without a solvent (the EFGWB Oneliner indicates methyl parathion water solubility is 60 ppm; The Merck Index indicates that the water solubility was 50 ppm; and the Pesticide Manual issued by the British Crop Council indicates that at 25°C the water solubility is 55 to 60 ppm).

**12. GOOD LABORATORY PRACTICE (GLP)/  
QUALITY ASSURANCE (QA) STATEMENTS**

GLP - signed yes

QA - signed yes

**13. COMPLETION OF ONE-LINER FOR STUDY:**

One-liner completed 9/30/94

Figure 1

Because D.O. monitoring was done on a 7 day basis, number of days the D.O. below 50% was estimated. The following is the method used to make that estimate:

Information and Assumptions:

- 1) Day 14 D.O. was 63.36%
- 2) Day 21 D.O. was 31.11%
- 3) Assumption is that the loss of D.O. is linear

The difference between Day 14 and minimal acceptable level (50%).  
 $63.36\% - 50.0\% = 13.6\%$

The loss from Day 14 and to Day 21.  
 $63.36\% - 31.11\% = 32.25\%$

The ratio between the 50% difference level and the loss over 7 days.  
 $13.6\% / 32.23\% = 0.42$

Number of days between represented by the 0.42 ratio.  
 $7 \text{ days} \times 0.42 = 2.9 \text{ days}$

Study day were the D.O. dropped to 50%.  
 $14 \text{ day} + 2.9 \text{ days} = 16.9 \text{ days}$

TABLE IV

Percent Survival<sup>a</sup> of *Daphnia magna* Exposed to Methyl Parathion for 21 Days During the Chronic Toxicity Study

Chamber ID (Nominal Conc.)	Mean Measured Conc. (µg/L)	Rep.	Initial Number Instar	Adult Surv.	Percent Surv.	Mean ± SD
Control	N/A	A	10	9	90	97.5 ± 5.0
		B	10	10	100	
		C	10	10	100	
		D	10	10	100	
Vehicle Blank	N/A	A	10	10	100	87.5 ± 9.6
		B	10	8	80	
		C	10	9	90	
		D	10	8	80	
Pooled Controls <sup>b</sup>						92.5 ± 8.9
Level 1 (0.12 µg/L)	0.076	A	10	10	100	100 ± 0.0
		B	10	10	100	
		C	10	10	100	
		D	10	10	100	
Level 2 (0.24 µg/L)	0.23	A	10	10	100	97.5 ± 5.0
		B	10	9	90	
		C	10	10	100	
		D	10	10	100	
Level 3 (0.50 µg/L)	0.43	A	10	10	100	92.5 ± 9.6
		B	10	8	80	
		C	10	9	90	
		D	10	10	100	
Level 4 (1.0 µg/L)	0.85	A	10	7	70	80 ± 12*
		B	10	7	70	
		C	10	9	90	
		D	10	9	90	
Level 5 <sup>c</sup> (2.0 µg/L)	1.7	A	10	0	0	0.0 ± 0.0*
		B	10	0	0	
		C	10	0	0	
		D	10	0	0	

\* Denotes values significantly different ( $p \leq 0.05$ ) from the pooled controls

<sup>a</sup> Data were subjected to frequency analysis coupled with a one-tailed Fisher's exact test.

<sup>b</sup> Control and vehicle blank were compared by a Student *t* test. A significant difference was not determined so comparison was made with the test levels using the pooled control value.

<sup>c</sup> Since this level was affected for survival, it was not included in the statistical analysis for all other parameters.

TABLE V

Young/Adult Reproduction Days and Time to First Brood of *Daphnia magna*  
Exposed to Methyl Parathion for 21 Days During the Chronic Toxicity Study

Chamber ID (Nominal Conc.)	Mean Measured Conc. (µg/L)	Rep.	Day 21 Reproduction <sup>a,b</sup>			Time to First Brood <sup>a,c</sup>		
			Total Young	Adult Reprod. Days	Young/ Adult Reprod. Days	Mean ± SD	Days	Mean ± SD (Days)
Control	N/A	A	980	149	6.58	7.83 ± 0.97	7	7.0 ± 0.0
		B	1314	150	8.76		7	
		C	1137	150	7.58		7	
		D	1259	150	8.39		7	
Vehicle Blank	N/A	A	1924	150	12.83	13.0 ± 0.24	7	7.0 ± 0.0
		B	1884	148	12.73		7	
		C	1928	146	13.21		7	
		D	1648	125	13.18		7	
Pooled Controls			—			7.0 ± 0.0		
Level 1 (0.12 µg/L)	0.076	A	1491	150	9.94	9.36 ± 1.5*	7	7.0 ± 0.0
		B	1580	150	10.53		7	
		C	1468	150	9.79		7	
		D	1075	150	7.17		7	
Level 2 (0.24 µg/L)	0.23	A	1788	150	11.92	11.5 ± 0.3*	7	7.0 ± 0.0
		B	1662	148	11.23		7	
		C	1705	150	11.37		7	
		D	1705	150	11.37		7	
Level 3 (0.50 µg/L)	0.43	A	1769	150	11.79	12.2 ± 0.71	7	7.0 ± 0.0
		B	1545	134	11.53		7	
		C	1774	135	13.14		7	
		D	1823	150	12.15		7	
Level 4 (1.0 µg/L)	0.85	A	1136	105	10.82	10.6 ± 0.38*	7	7.0 ± 0.0
		B	1155	105	11.00		7	
		C	1404	135	10.40		7	
		D	1374	135	10.18		7	
Level 5 (2.0 µg/L)	1.7	A	276	36	7.67	7.50 ± 1.3 <sup>d</sup>	7	7.0 ± 0.0 <sup>d</sup>
		B	288	31	9.29		7	
		C	379	55	6.89		7	
		D	240	39	6.15		7	

\* Denotes values significantly different ( $p \leq 0.05$ ) from the vehicle blank

- d Data were subjected to a one-way analysis of variance and Dunnett's multiple means comparison test.
- b Control and vehicle blank were compared by a Student *t*-test. A significant difference was determined so comparison was made with the test levels using the vehicle blank.
- e Control and vehicle blank were compared by a Student *t*-test. No significant difference was determined so comparison was made with the test levels using the pooled controls.
- d Since this level was affected for survival, it was excluded from statistical analysis for this parameter.



TABLE VI

Adult Weights and Length Measurements of *Daphnia magna* Exposed to Methyl Parathion for 21 Days During the Chronic Toxicity Study

Chamber ID (Nominal Conc.)	Mean Measured Conc. ( $\mu\text{g/L}$ )	Rep.	Adult Weights ( $\text{mg}$ ) <sup>a,b</sup>				Day 21 Daphnid Length ( $\text{mm}$ ) <sup>a,b</sup>	
			Adult Surv.	Total Weight	Average Daphnid Weight	Mean $\pm$ SD	Mean Length	Mean $\pm$ SD
Control	N/A	A	9	2.4	0.27		3.57	
		B	10	6.1	0.61	$0.47 \pm 0.14$	3.91	$3.83 \pm 0.18$
		C	10	5.1	0.51		3.96	
		D	10	4.7	0.47		3.91	
Vehicle Blank	N/A	A	10	7.2	0.72	$0.78 \pm 0.06$	4.35	$4.44 \pm 0.07$
		B	8	5.8	0.73		4.43	
		C	9	7.3	0.81		4.50	
		D	8	6.8	0.85		4.49	
Level 1 (0.12 $\mu\text{g/L}$ )	0.076	A	10	6.7	0.67	$0.64 \pm 0.10$	4.21	$4.11 \pm 0.17^*$
		B	10	7.0	0.70		4.23	
		C	10	7.1	0.71		4.14	
		D	10	4.9	0.49		3.86	
Level 2 (0.24 $\mu\text{g/L}$ )	0.23	A	10	8.0	0.80	$0.77 \pm 0.09$	4.40	$4.33 \pm 0.06$
		B	9	5.8	0.64		4.28	
		C	10	8.3	0.83		4.28	
		D	10	8.0	0.80		4.37	
Level 3 (0.50 $\mu\text{g/L}$ )	0.43	A	10	6.5	0.65	$0.70 \pm 0.07$	4.41	$4.42 \pm 0.04$
		B	8	5.1	0.64		4.39	
		C	9	6.2	0.69		4.47	
		D	10	8.0	0.80		4.42	
Level 4 (1.0 $\mu\text{g/L}$ )	0.85	A	7	5.1	0.73	$0.62 \pm 0.08^*$	4.26	$4.28 \pm 0.02^*$
		B	7	3.8	0.54		4.27	
		C	9	5.5	0.61		4.27	
		D	9	5.2	0.58		4.31	
Level 5 <sup>c</sup> (2.0 $\mu\text{g/L}$ )	1.7	A	0	—	—	—	—	—
		B	0	—	—	—	—	—
		C	0	—	—	—	—	—
		D	0	—	—	—	—	—

\* Denotes values significantly different ( $p \leq 0.05$ ) from the vehicle blank

a Data were subjected to a one-way analysis of variance and Dunnett's multiple means comparison test.

b Control and vehicle blank were compared by a Student *t*-test. A significant difference was determined so comparison with the test levels were made using the vehicle blank.

c Since this level was affected for survival, it was not included in the statistical analysis for this parameter.

Methyl Parathion 21 day Daphnia magna Study-Length

File: c:\chem\ch3para\ch3parl2.dat

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	18792.652	4698.163	1.308
Within (Error)	178	639510.015	3592.753	
Total	182	658302.667		

Critical F value = 2.45 (0.05,4,120)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ :All groups equal

Methyl Parathion 21 day Daphnia magna Study-Length

File: c:\chem\ch3para\ch3parl2.dat

Transform: NO TRANSFORM

BONFERRONI T-TEST

TABLE 1 OF 2

$H_0$ :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Sol. Control	88.771	88.771		
2	0.12	82.150	82.150	0.477	
3	0.24	86.667	86.667	0.151	
4	0.5	110.324	110.324	-1.525	
5	1.0	85.531	85.531	0.221	

Bonferroni T table value = 2.27 (1 Tailed Value,  $P=0.05$ ,  $df=120,4$ )

Methyl Parathion 21 day Daphnia magna Study-Length

File: c:\chem\ch3para\ch3parl2.dat

Transform: NO TRANSFORM

BONFERRONI T-TEST

TABLE 2 OF 2

$H_0$ :Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Sol. Control	35			
2	0.12	40	31.492	35.5	6.621
3	0.24	39	31.680	35.7	2.105
4	0.5	37	32.083	36.1	-21.553
5	1.0	32	33.279	37.5	3.240

Methyl Parathion 21 day Daphnia magna Study-Length

File: c:\chem\ch3para\ch3par12.dat

Transform: NO TRANSFORM

## ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	18792.652	4698.163	1.308
Within (Error)	178	639510.015	3592.753	
Total	182	658302.667		

Critical F value = 2.45 (0.05,4,120)

Since F &lt; Critical F FAIL TO REJECT Ho:All groups equal

Methyl Parathion 21 day Daphnia magna Study-Length

File: c:\chem\ch3para\ch3par12.dat

Transform: NO TRANSFORM

## DUNNETTS TEST

\*\*\*\*\* WARNING \*\*\*\*\*

This data set has unequal replicates. The Bonferroni T-test should be used instead of the Dunnetts test.

Methyl Parathion 21 day Daphnia magna Study-Length

File: c:\chem\ch3para\ch3par12.dat

Transform: NO TRANSFORM

## DUNNETTS TEST - TABLE 1 OF 2

Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Sol. Control	88.771	88.771		
2	0.12	82.150	82.150	0.477	
3	0.24	86.667	86.667	0.151	
4	0.5	110.324	110.324	-1.525	
5	1.0	85.531	85.531	0.221	

Dunnett table value = 2.18 (1 Tailed Value, P=0.05, df=120,4)

Methyl Parathion 21 day Daphnia magna Study-Length

File: c:\chem\ch3para\ch3par12.dat

Transform: NO TRANSFORM

## DUNNETTS TEST - TABLE 2 OF 2

Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Sol. Control	35			
2	0.12	40	30.244	34.1	6.621
3	0.24	39	30.424	34.3	2.105
4	0.5	37	30.811	34.7	-21.553
5	1.0	32	31.959	36.0	3.240

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	4	6.580	8.760	7.823
2	Sol. Control	4	12.730	13.210	12.988
3		0.12	7.170	10.530	9.357
4		0.24	11.230	11.920	11.473
5		0.5	11.530	13.140	12.153
6		1.0	10.180	11.000	10.600
7		2.0	6.150	9.290	7.500

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
File: ch3payad Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.938	0.968	0.484
2	Sol. Control	0.059	0.243	0.122
3		2.229	1.493	0.746
4		0.093	0.306	0.153
5		0.498	0.706	0.353
6		0.142	0.376	0.188
7		1.809	1.345	0.673

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
File: ch3payad Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	107.917	17.986	21.828
Within (Error)	21	17.304	0.824	
Total	27	125.221		

Critical F value = 2.57 (0.05, 6, 21)  
Since P > Critical F REJECT Ho: All groups equal

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
File: ch3payad Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2					Ho: Control < Treatment	
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN	T STAT	SIG	
1	Control	7.823				
2	Sol. Control	12.988				
3		9.357				
4		11.473				
5		12.153				
6		10.600				

7  
Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20, 6)

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
File: ch3payad Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2					Ho: Control < Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum sig Diff (IN ORIG. UNITS)	CONTROL	DIFFERENCE FROM CONTROL	
1	Control	4				
2	Sol. Control	4	1.579	20.2	-6.165	
3		4	1.579	20.2	-1.535	
4		4	1.579	20.2	-3.650	
5		4	1.579	20.2	-4.330	
6		4	1.579	20.2	-2.778	
7		4	1.579	20.2	0.323	

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
File: ch3payad Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2						
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN	
1	Control	4	7.823	7.823	10.759	
2	Sol. Control	4	12.988	12.988	10.759	
3		4	9.357	9.357	10.759	
4		4	11.473	11.473	10.759	
5		4	12.153	12.153	10.759	
6		4	10.600	10.600	10.600	
7		4	7.500	7.500	7.500	

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
File: ch3payad Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2						
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=0.05	TABLE WILLIAMS	DEGREES OF FREEDOM	
Control	10.759					
Sol. Control	10.759	4.574	*	1.72	k=1, v=21	
	0.12	4.574	*	1.80	k=2, v=21	
	0.24	4.574	*	1.83	k=3, v=21	
	0.5	4.574	*	1.84	k=4, v=21	
	1.0	4.327	*	1.85	k=5, v=21	
	2.0	0.502		1.85	k=6, v=21	

S = 0.908

Note: df used for table values are approximate when v > 20.

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
 File: c:\chem\ch3para\ch3pyad2 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	79.955	15.991	19.865
Within (Error)	18	14.490	0.805	
Total	23	94.445		

Critical F value = 2.77 (0.05,5,18)  
 Since F > Critical F REJECT Ho:All groups equal

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
 File: c:\chem\ch3para\ch3pyad2 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Sol. Control	12.988	12.988		
2	0.12	9.357	9.357	5.722	*
3	0.24	11.473	11.473	2.388	
4	0.5	12.153	12.153	1.316	
5	1.0	10.600	10.600	3.763	*
6	2.0	7.500	7.500	8.650	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
 File: c:\chem\ch3para\ch3pyad2 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Sol. Control	4			
2	0.12	4	1.529	11.8	3.630
3	0.24	4	1.529	11.8	1.515
4	0.5	4	1.529	11.8	0.835
5	1.0	4	1.529	11.8	2.387
6	2.0	4	1.529	11.8	5.488



Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
 File: c:\chem\ch3para\ch3pyad3 Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	31.466	7.866	13.023
Within (Error)	15	9.063	0.604	
Total	19	40.529		

Critical F value = 3.06 (0.05,4,15)  
 Since F > Critical F REJECT Ho:All groups equal

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
 File: c:\chem\ch3para\ch3pyad3 Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Sol. Control	12.988	12.988		
2	0.12	9.357	9.357	6.605	*
3	0.24	11.473	11.473	2.757	*
4	0.5	12.153	12.153	1.519	
5	1.0	10.600	10.600	4.345	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

Methyl Parathion 21 Day Daphnia magna - Young/Adult/Day  
 File: c:\chem\ch3para\ch3pyad3 Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Sol. Control	4			
2	0.12	4	1.297	10.0	3.630
3	0.24	4	1.297	10.0	1.515
4	0.5	4	1.297	10.0	0.835
5	1.0	4	1.297	10.0	2.387

TITLE: Percent Survival of Daphnia magna CH3 Parathion 21 Days  
FILE: c:\chem\iprodion\survival.dat  
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	9.0000	9.0000
1	Control	2	10.0000	10.0000
1	Control	3	10.0000	10.0000
1	Control	4	10.0000	10.0000
2	Sol. Control	1	10.0000	10.0000
2	Sol. Control	2	8.0000	8.0000
2	Sol. Control	3	9.0000	9.0000
2	Sol. Control	4	8.0000	8.0000
3	0.12	1	10.0000	10.0000
3	0.12	2	10.0000	10.0000
3	0.12	3	10.0000	10.0000
3	0.12	4	10.0000	10.0000
4	0.24	1	10.0000	10.0000
4	0.24	2	9.0000	9.0000
4	0.24	3	10.0000	10.0000
4	0.24	4	10.0000	10.0000
5	0.50	1	10.0000	10.0000
5	0.50	2	8.0000	8.0000
5	0.50	3	9.0000	9.0000
5	0.50	4	10.0000	10.0000
6	1.0	1	7.0000	7.0000
6	1.0	2	7.0000	7.0000
6	1.0	3	9.0000	9.0000
6	1.0	4	9.0000	9.0000
7	2.0	1	0.0000	0.0000
7	2.0	2	0.0000	0.0000
7	2.0	3	0.0000	0.0000
7	2.0	4	0.0000	0.0000

Percent Survival of Daphnia magna CH3 Parathion 21 Days  
File: c:\chem\iprodion\survival.dat Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	4	9.000	10.000	9.750
2	Sol. Control	4	8.000	10.000	8.750
3	0.12	4	10.000	10.000	10.000
4	0.24	4	9.000	10.000	9.750
5	0.50	4	8.000	10.000	9.250
6	1.0	4	7.000	9.000	8.000
7	2.0	4	0.000	0.000	0.000

Percent Survival of Daphnia magna CH3 Parathion 21 Days  
 File: c:\chem\iprodion\survival.dat Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.250	0.500	0.250
2	Sol. Control	0.917	0.957	0.479
3	0.12	0.000	0.000	0.000
4	0.24	0.250	0.500	0.250
5	0.50	0.917	0.957	0.479
6	1.0	1.333	1.155	0.577
7	2.0	0.000	0.000	0.000

Percent Survival of Daphnia magna CH3 Parathion 21 Days  
 File: c:\chem\iprodion\survival.dat Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	9.750	9.750	9.750
2	Sol. Control	4	8.750	8.750	9.500
3	0.12	4	10.000	10.000	9.500
4	0.24	4	9.750	9.750	9.500
5	0.50	4	9.250	9.250	9.250
6	1.0	4	8.000	8.000	8.000
7	2.0	4	0.000	0.000	0.000

Percent Survival of Daphnia magna CH3 Parathion 21 Days  
 File: c:\chem\iprodion\survival.dat Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	9.750				
Sol. Control	9.500	0.489		1.72	k= 1, v=21
0.12	9.500	0.489		1.80	k= 2, v=21
0.24	9.500	0.489		1.83	k= 3, v=21
0.50	9.250	0.977		1.84	k= 4, v=21
1.0	8.000	3.420	*	1.85	k= 5, v=21
2.0	0.000	19.052	*	1.85	k= 6, v=21

s = 0.724

Note: df used for table values are approximate when v > 20.

TITLE: Methyl Parathion 21 Day Daphnia magna Study - Weight  
FILE: c:\chem\ch3para\dafwt.dat  
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 5

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Sol.Control	1	7.2000	7.2000
1	Sol.Control	2	5.8000	5.8000
1	Sol.Control	3	7.3000	7.3000
1	Sol.Control	4	6.8000	6.8000
2	0.12	1	6.7000	6.7000
2	0.12	2	7.0000	7.0000
2	0.12	3	7.1000	7.1000
2	0.12	4	4.9000	4.9000
3	0.24	1	8.0000	8.0000
3	0.24	2	5.8000	5.8000
3	0.24	3	8.3000	8.3000
3	0.24	4	8.0000	8.0000
4	0.5	1	6.5000	6.5000
4	0.5	2	5.1000	5.1000
4	0.5	3	6.2000	6.2000
4	0.5	4	8.0000	8.0000
5	1.0	1	5.1000	5.1000
5	1.0	2	3.8000	3.8000
5	1.0	3	5.5000	5.5000
5	1.0	4	5.2000	5.2000

Methyl Parathion 21 Day Daphnia magna Study - Weight  
File: c:\chem\ch3para\dafwt.dat Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Sol.Control	4	5.800	7.300	6.775
2	0.12	4	4.900	7.100	6.425
3	0.24	4	5.800	8.300	7.525
4	0.5	4	5.100	8.000	6.450
5	1.0	4	3.800	5.500	4.900

Methyl Parathion 21 Day Daphnia magna Study - Weight  
File: c:\chem\ch3para\dafwt.dat Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Sol.Control	0.469	0.685	0.342

2	0.12	1.062	1.031	0.515
3	0.24	1.342	1.159	0.579
4	0.5	1.430	1.196	0.598
5	1.0	0.567	0.753	0.376

Methyl Parathion 21 Day Daphnia magna Study - Weight

File: c:\chem\ch3para\dafwt.dat

Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	14.633	3.658	3.756
Within (Error)	15	14.613	0.974	
Total	19	29.246		

Critical F value = 3.06 (0.05,4,15)

Since F > Critical F REJECT Ho:All groups equal

Methyl Parathion 21 Day Daphnia magna Study - Weight

File: c:\chem\ch3para\dafwt.dat

Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Sol.Control	6.775	6.775		
2	0.12	6.425	6.425	0.502	
3	0.24	7.525	7.525	-1.075	
4	0.5	6.450	6.450	0.466	
5	1.0	4.900	4.900	2.687	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

Methyl Parathion 21 Day Daphnia magna Study - Weight

File: c:\chem\ch3para\dafwt.dat

Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Sol.Control	4			
2	0.12	4	1.647	24.3	0.350
3	0.24	4	1.647	24.3	-0.750
4	0.5	4	1.647	24.3	0.325
5	1.0	4	1.647	24.3	1.875

Methyl Parathion 21 Day Daphnia magna Study - Weight

File: c:\chem\ch3para\dafwt.dat

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol.Control	4	6.775	6.775	6.908
2	0.12	4	6.425	6.425	6.908
3	0.24	4	7.525	7.525	6.908
4	0.5	4	6.450	6.450	6.450
5	1.0	4	4.900	4.900	4.900

Methyl Parathion 21 Day Daphnia magna Study - Weight

File: c:\chem\ch3para\dafwt.dat

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol.Control	6.908				
0.12	6.908	0.191		1.75	k= 1, v=15
0.24	6.908	0.191		1.84	k= 2, v=15
0.5	6.450	0.466		1.87	k= 3, v=15
1.0	4.900	2.687	*	1.88	k= 4, v=15

s = 0.987

Note: df used for table values are approximate when v > 20.



Percent D.O. 21 Day Daphnia Study											
	Day 0	%	Day 4	%	Day 7	%	Day 14	%	Day 21	%	
Control	8.1	93.32%	8	92.17%	7.8	91.44%	8.2	94.47%	8	92.17%	
Sol. Contr	6.2	71.43%	5.4	62.21%	6.2	72.68%	5.7	65.67%	4.9	56.45%	
0.12	7.6	87.56%	7.6	87.56%	7.6	89.10%	7.8	89.86%	7.6	87.56%	
0.24	7.1	81.80%	6.7	77.19%	6.8	79.72%	6.7	77.19%	6.9	79.49%	
0.5	6.2	71.43%	6.4	73.73%	5.8	68.00%	6.1	70.28%	4.7	54.15%	
1	6.4	73.73%	6.3	72.58%	5.8	68.00%	4.8	55.30%	4.4	50.69%	
2	5.7	65.67%	5.2	59.91%	5.3	62.13%	5.5	63.36%	2.7	31.11%	

DO GRAT

# Methyl Parathion 21-Day Daphnia Study D.O. for Day 14 and Day 21

